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Review article

Prenatal programming of neuroendocrine reproductive function

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ABSTRACT

It is now well recognized that the gestational environment can have long-lasting effects not only on the life span and health span of an individual but also, through potential epigenetic changes, on future generations. This article reviews the "prenatal programming" of the neuroendocrine systems that regulate reproduction, with a specific focus on the lessons learned using ovine models. The review examines the critical roles played by steroids in normal reproductive development before considering the effects of prenatal exposure to exogenous steroid hormones including androgens and estrogens, the effects of maternal nutrition and stress during gestation, and the effects of exogenous chemicals such as alcohol and environment chemicals. In so doing, it becomes evident that, to maximize fitness, the regulation of reproduction has evolved to be responsive to many different internal and external cues and that the GnRH neurosecretory system expresses a degree of plasticity throughout life. During fetal life, however, the system is particularly sensitive to change and at this time, the GnRH neurosecretory system can be "shaped" both to achieve normal sexually differentiated function but also in ways that may adversely affect or even prevent "normal function". The exact mechanisms through which these programmed changes are brought about remain largely uncharacterized but are likely to differ depending on the factor, the timing of exposure to that factor, and the species. It would appear, however, that some afferent systems to the GnRH neurons such as kisspeptin, may be critical in this regard as it would appear to be sensitive to a wide variety of factors that can program reproductive function. Finally, it has been noted that the prenatal programming of neuroendocrine reproductive function can be associated with epigenetic changes, which would suggest that in addition to direct effects on the exposed offspring, prenatal programming could have transgenerational effects on reproductive potential.

1. Introduction

It is now well recognized that an animal's adult phenotype is the result of both genetic processes and the prenatal environment, which can be influenced by maternal conditions not only during pregnancy but also before conception. Although potentially an adaptive strategy, whether this is for the benefit of the fetus, to ensure that it is "matched" to its postnatal environment, or to maximize maternal fitness, is controversial. The concept that the gestational environment shapes offspring phenotype has been well described with regard to energy partitioning and/or metabolism and the resultant "thrifty phenotype" of offspring where maternal nutrition has been restricted [1]. Such "prenatal programming," is associated with functional changes in neural control systems and has been shown to be applicable to other physiological systems

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including reproduction. Normal mammalian development is characterized by a series of "critical periods," during which the fetal environment can influence postnatal form and function, via plastic changes in neural and neuroendocrine systems. A classic example of developmental plasticity occurs during normal sexual differentiation of the neuroendocrine regulatory systems controlling mammalian reproduction. Normal sexual differentiation of the brain is regulated by testosterone, secreted by the testes, during the neonatal period in rodents but during the prenatal period in humans and sheep [2]. On conception, the resultant embryo is by default phenotypically female; however, under the direction of the SRY gene on the Y chromosome, the production of testosterone results in regression of the female genital tract and development of the male reproductive organs and the programming of a permanent "male" phenotype. This includes plastic changes at the level of the neuroendocrine hypothalamus, such that the male phenotype loses the ability of estradiol to induce a GnRH and/or LH surge (i.e., estradiol positive feedback), which is one of the defining features of female reproductive physiology among spontaneously ovulating mammals. Having established that the reproductive axis exhibits such a large degree of developmental plasticity, it is not surprising that additional developmental influences such as stress, nutrition, and toxins, can also affect reproductive development. In this article, we review the effects of androgens, nutrition, and toxins, including alcohol and environmental chemicals (ECs), and the effects of prenatal stress on reproduction with a focus on the neuroendocrine mechanisms that are implicated in the observed effects on phenotype and the results obtained from ovine models.

2. Plasticity is normal within the reproductive system

Normal reproductive function relies on the coordinated production of gametes and the expression of behavior to facilitate fertilization. These critical reproductive system functions are regulated by the delicately coordinated actions of steroid hormones synthesized in the male and female gonads. Gonadal steroid secretion is regulated by the pituitary gonadotrophins, LH, and FSH, the secretion of which is directed by the patterned release of the hypothalamic neuropeptide GnRH [3–6]. Changes in the patterns of GnRH secretion occur at key reproductive transitions, including puberty, the estrous and/or menstrual cycle, menopause, and the annual reproductive cycle in seasonal breeding animals. These changes in the pattern of GnRH secretion are associated with markers of neuronal plasticity and suggest that remodeling of GnRH neurons may occur at these times [7,8]. GnRH secretion, and therefore reproductive function, can also be influenced by internal factors, such as nutritional state, pregnancy, and stress. The ability of these diverse regulatory inputs to impact on the reproductive system occurs via a series of afferent neuronal inputs to the GnRH neurons, which collectively with the GnRH neurons are often referred to as the "GnRH neurosecretory system". Within rodents and sheep, GnRH neuronal cell bodies are predominantly located in the ventral preoptic area (POA) with a small additional population present in the mediobasal hypothalamus [9,10], [11] whereas in primates, guinea pigs, and rabbits, most GnRH cell bodies are located within the mediobasal hypothalamus. In each case, the axons of the GnRH neurons extend to the median eminence [12,13], and dendritic connections encompass additional brain areas including the POA and arcuate nucleus (ARC) where afferent systems such as noradrenaline [14], kisspeptin, dynorphin, neurokinin B (KNDy neurons) [15], gamma aminobutyric acid, neuropeptide Y, agouti related peptide, pro-opiomelanocortin [16], and cocaine- and amphetamine-regulated transcript are found, which are known to regulate GnRH release. Thus, plasticity within the reproductive system can occur through changes in neuronal connectivity within the GnRH neurosecretory system. The following section will summarize effects of some of the major factors known to program the reproductive neuroendocrine axis before birth.

3. Effects of prenatal exposure to exogenous steroid hormones

3.1. Androgens

Prenatal exposure to testosterone is a critical component of normal sexual differentiation in mammals. A number of animal models have been developed in which exogenous testosterone (or other androgens such as dihydrotestosterone) have been administered during gestation to examine the developmental effects of androgen exposure and the physiological mechanisms through which specific effects are mediated. This section of the review will concentrate on the substantial studies that have been conducted on the effects of prenatal testosterone exposure in the sheep and the developmental windows that are key to programming postnatal reproductive development. These studies show that exposure of female fetuses to concentrations of androgens similar to that observed in male fetuses between days 30 and 90 of a 147-day gestation results in complete virilization of the external genitalia, and the presence of internal male structures such as the bulbourethral glands. This exposure pattern also results in advanced puberty, altered estrous cyclicity [17,18] followed by anovulation during the second breeding season [17], and decreased primordial follicle number [17,19] but a multifollicular ovarian phenotype [19,20]. Although this treatment does not result in fetal exposure to pharmacologic concentrations of steroid hormones, the reproductive axis of male lambs born to testosterone treated ewes is also affected, specifically, they exhibit reduced scrotal circumference, higher numbers of sertoli cells [21], and reduced sperm count and motility [22]. Similar effects have been noted after *in utero* androgen exposure in other species including monkey [23–27] rat [28,29], and mouse [30].

3.1.1. Do prenatal androgens program the reproductive neuroendocrine system?

The neuroendocrine effects of gestational androgen exposure are perhaps the best characterized in the ovine model, with alterations being seen at all levels of the reproductive axis from the GnRH neurons and afferents to the level of the pituitary gland and the gonads. The

testosterone-induced changes in the gonads are accompanied by hypersecretion of LH in both genders [31,32], which is accompanied by increased testosterone secretion in the males [31]. In the females, all aspects of the normal homeostatic steroidogenic feedback regulation of GnRH and/or LH secretion appear to be disturbed, i.e., the animals show reduced sensitivity to estradiol [33,34] and progesterone negative feedback [35] and express either significantly reduced [36] [33,37,38] or no [37] estradiol positive feedback. It is well accepted that GnRH neurons lack classical estrogen receptors (estrogen receptor alpha, ERα); therefore, steroidal feedback onto GnRH neurons is via afferent systems, which express receptors such as ERα and are sensitive to steroid feedback from the gonads. It is now widely accepted that KNDy (kisspeptin/neurokinin B/dynorphin) neurons, the cell bodies of which are located in the ARC are critical for the negative feedback actions of estradiol on pulsatile LH secretion and that the ARC KNDy neurons, in association with KNDy neurons from the POA, are involved in estradiol positive feedback [39]. Prenatal testosterone exposure is associated with decreased dynorphin and NKB content in ARC KNDy neurons [40], but at the level of the POA, an increase in KNDy cell somas size and a decrease in synaptic connectivity onto both KNDy and Kisspeptin neurons [41] suggests differential effects on these two populations of cells. The observed changes in these two populations of cells is potentially important mechanistically, as evidence suggests that the ARC KNDy neurons are critical for the expression of progesterone negative feedback [42–44], and that KNDy autoregulation via NK3R signaling is involved in both estradiol negative and positive feedback. Furthermore, NK3R expression is also reduced in the ARC of *in utero* androgenized female sheep [45]. Finally, prenatal testosterone exposure is associated with a reduction in the synaptic inputs to GnRH neurons, and this is thought to be due to a specific reduction in innervation by KNDy neurons [41,46].

3.2. Estrogens

Much less has been published with regard to experimental programming effects of prenatal estrogen and progesterone exposure. Studies in mice have indicated that normal patterns of prenatal estrogen exposure are important for sexual differentiation of the mouse brain and that abnormal exposure may specifically affect the kisspeptin and/or GnRH neurosecretory system [47]. The effects of prenatal estrogen exposure are thereafter supplemented by studies in rodents that have documented the effects of prenatal exposure to the synthetic estrogen diethylstilbestrol (DES) [48–52] and human medicine, where there are numerous studies that have characterized the effects of administration of DES after its use, clinically, to support pregnancies [53]. Although the most obvious effects of gestational DES exposure are teratogenic, female exposure to DES, *in utero*, is also associated with an increased risk of infertility, reproductive cancers, spontaneous abortion, preterm delivery, loss of second-trimester pregnancy, ectopic pregnancy, preeclampsia, stillbirth, neonatal death, early menopause, and breast cancer, indicating multiple deficits in the reproductive system [53]. These outward manifestations of reproductive disruption are accompanied in adult women by lower concentrations of estradiol and inhibin B and higher concentrations of FSH and LH [54], suggesting central effects of prenatal DES exposure. In males, prenatal DES exposure is associated with testicular dysgenesis syndrome and its component conditions including epididymal cysts, hypospadias, cryptorchidism, and low semen quality [55,56]. These deficits are associated with altered gonadotrophin secretion and pituitary cell populations in ovine studies [57,58] and altered testicular development, sperm function, and steroid signaling in rats [59]. Perplexingly, the effects of prenatal progesterone exposure have not been documented despite the extensive use of progestagenic compounds to prevent preterm labor in human medicine [60] or in contraceptives used during lactation (World Health Organization, 2010. Medical Eligibility Criteria for Contraceptive Use, 4th Ed. Geneva [www.who.int/reproductivehealth/publications]).

4. Programming effects of maternal nutrition during gestation

The ability to link reproductive function and nutrition and/or energy balance provides a means to ensure that if resources are scant, they can be dedicated to survival, but when capacity exists, used for reproduction. As indicated previously, the pioneering work of Barker et al. has proven that nutrition programs the metabolic phenotype in adulthood. It has become apparent, however, that the effects are not limited to metabolic regulation and that prenatal and/or developmental nutrition can also affect later reproductive function. The effects observed are complicated, however, as they can be sexually differentiated and both overnutrition and undernutrition can be associated with similar and/or opposing effects and be species-specific. Furthermore, the effects are influenced by the context in which the nutritional manipulation is imposed including the age and/or growth status of the mother. Our understanding of the neuroendocrine systems regulating energy balance has expanded greatly in the last 25 years with the identification of a host of regulatory neuropeptides including leptin, ghrelin, PYY, and CCK, many of which can communicate with and, therefore, provide a link between nutrition and the GnRH neurosecretory system [61]. As some of these energy signaling systems can have antagonistic actions on satiety and hunger, and can be influenced by the composition as well as the caloric content of ingested food, this provides a mechanism for fine control of the interplay between reproduction and nutrition. In addition, it provides a neuroendocrine system that could be subject to prenatal programming although this has not yet been well characterized.

4.1. Effects of maternal undernutrition

In sheep, maternal undernutrition during pregnancy has been reported to be without effect on the timing of female puberty [62,63] although it is associated with reduced fertility [64], a reduction in fetal ovarian mass, a delay in germ cell maturation and the onset of meiosis [65], and altered follicular dynamics [63]. In male sheep,

although some reports also indicate that undernutrition does not alter the timing of puberty [66], others report a delay [62] and an inhibitory effect on mean Sertoli cell number and seminiferous tubules diameter [66]. These results are supported by studies in other species, e.g., with regard to the female, in both humans [67,68] and rats [69], maternal undernutrition has been linked with reduced fertility and an increased risk of cystic ovarian structures and, in rats, altered ovarian follicular dynamics [70]. Similarly, in male rats, developmental nutrient restriction is associated with delayed puberty [70] and reduced seminiferous tubule diameter [70,71], and gestational protein restriction is associated with reduced Sertoli cell number, sperm count and motility and increased morphologic testicular and sperm abnormalities [71–73].

4.1.1. Does maternal undernutrition program the reproductive neuroendocrine system?

The observed effects suggest that maternal undernutrition does affect the reproductive neuroendocrine system [70,74], but the diverse nature of the effects suggests that either multiple systems are involved or effects are present at multiple levels within the reproductive axis. Studies in sheep have shown that maternal undernutrition is associated with altered pituitary responsiveness to GnRH, both with regard to LH [75] and FSH [63,66] release. Studies in rats have shown increased basal gonadotrophin levels after maternal undernutrition, which would suggest that this manipulation programmed either increased pituitary sensitivity to GnRH or increased GnRH drive [70,76]. In addition, it was noted that rats born to mothers subjected to undernutrition during gestation also had altered sensitivity of the reproductive axis, specifically gonadotrophin secretion to reduced food intake, although these effects appear to be attributable to altered serum leptin concentrations as opposed to specific effects at the level of the hypothalamus [76]. That said, it has been reported that, in rats, maternal undernutrition results in premature reproductive senescence, which could be due to changes in hypothalamic function as it is accompanied by altered hypothalamic estradiol receptor alpha expression [69].

4.2. Effects of maternal overnutrition

Although maternal undernutrition could be predicted to be detrimental for offspring development and, thus, reproductive function, maternal overnutrition can also have adverse effects. In adolescent sheep, maternal overnutrition can result in intrauterine growth restriction (IUGR) as nutrients are used to promote maternal rather than fetal growth [77]. This form of IUGR, is associated with sexually differentiated effects on the timing of puberty; females being unaffected, whereas puberty is significantly delayed in males [62]. Although puberty was not affected in the females, ovine maternal overnutrition is associated with a reduction in both the total number of ovarian follicles and the number of primordial follicles [78] and ovarian function in sheep [62]. Conversely, IUGR as a consequence of maternal overnutrition has no effect on the number of Sertoli cells or seminiferous tubules in the fetal male testes [78] but is associated with reduced serum testosterone concentrations, smaller testicular volume, and a later seasonal increase in plasma testosterone in sheep after birth [62]. In rats, gestational overnutrition has a negative effect on the timing of puberty in both male and female offspring and results in irregular estrous cycles in female offspring [79,80] and retarded testicular growth in male rats [70,72].

4.2.1. Does maternal overnutrition program the reproductive neuroendocrine system?

Again although the changes in reproductive function noted previously are suggestive of changes in the neuroendocrine systems regulating reproductive function, very few studies have addressed the physiological mechanisms that might underlie the observed changes in function. Work using an ovine model has shown that overnutrition is associated with reduced prepubertal LH concentrations in males [62] but has no effects on pituitary LH beta or FSH beta mRNA expression or plasma gonadotrophin concentrations in either gender [78].

5. Effects of stress during gestation

Situations, such as undernutrition, can be perceived as a physiological threat and as such, in addition to changes in circulating metabolic signals, results in activation of the hypothalamo-pituitary-adrenal (HPA) axis. It is possible, therefore, that noted effects of undernutrition are mediated by activation of the physiological “stress” response and consequent changes in cortisol/corticotropin-releasing hormone (CRH) concentrations. The interaction between the stress response and reproduction, in the adult, has been extensively studied, in several species, as it is well known that exposure to a stressor (physical or psychological) can inhibit reproductive function. This cross talk between these two physiological systems most likely occurs within the hypothalamus, as specific neuronal systems are common to both the regulation of GnRH and CRH secretion, e.g., noradrenaline, neuropeptide Y, gamma aminobutyric acid, and serotonin [81]. In addition, glucocorticoid receptors are expressed on GnRH neurons [82]. However, despite the known interactions between the HPA and hypothalamo-pituitary-gonadal axes [81,83,84], the potential effects of increased CRH or cortisol or the activation of the HPA axis (maternal or fetal) by applied stressors (e.g., heat, handling, inflammation), on the prenatal development of the reproductive axis has not been extensively studied.

Administration of cortisol or the synthetic corticosteroid betamethasone to pregnant ewes [85] and rats [86–88] (RistiĆ et al., 2008) [89] has substantial effects on the offspring. In males, this includes effects on the gonads such as a reduction in the length of testicular cords, the amount of interstitial tissue and testicular weight [85], as well as reduced testosterone production [86,87], lowered sperm parameters, fertility [87] and reduced anogenital distance [89]. In females, gestational exposure to exogenous cortisol has been reported to be associated with a decrease in the number of ovarian follicles [90] and delayed vaginal opening [88]. A variety of effects on the reproductive systems of offspring have also been reported when a range of

different stressors were experienced by their mothers during gestation, these include; restraint stress, which results in demasculinization/feminization of behavior patterns in male rats [91,92]; overcrowding, which results in increased anogenital distance in female mice [93]; repeated exposure to bright lights, which reduces sexual behavior in male rats [89]; and exposure to an electromagnetic field (wireless internet frequency) for 1 hour/day, which can delay puberty in rats [94]. Prenatally heat stressed cattle exhibit decreased fertility, as measured by the number of matings required to achieve a successful pregnancy and thereafter exhibit a lower milk yield than "normal" animals (G.E. Dahl, Oral personal communication, 2015). Handling stress results in reduced gonadal weight in both male and female blue fox cubs and a reduction in anogenital distance in female offspring [95].

5.1. Does gestational stress program the reproductive neuroendocrine system?

Although the mechanisms behind these reported effects have not, in general, been well characterized, the accompanying decrease in basal estradiol and testosterone production in the blue foxes [95], lowered serum LH levels in rats after exposure to electromagnetic fields [94], and the loss of responsiveness in dopamine levels within the nucleus accumbens in prenatally stressed male rats in response to an estrus female [92] all suggest that the reproductive neuroendocrine systems in these animals have been affected by prenatal stress. This proposition is also supported by the recent observation that the presence of an inflammatory process in the mother induced by an intraperitoneal injection of lipopolysaccharide, which should also stimulate a stress response, is accompanied by a decrease in the number of GnRH neurons in the fetal forebrain [96].

6. Effects of exogenous chemical exposure during gestation

Within the natural, domestic, and work environment, animals and humans are surrounded by chemicals, many of which have been shown to impact on the normal functioning of physiological systems, in particular, the reproductive system. Some of these chemicals are natural, e.g., phytoestrogens and alcohol; however, others are of anthropogenic origin and include pharmaceuticals such as synthetic estrogens, industrial chemicals and their by-products, such as dioxins, polybrominated diphenyl ethers, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons, alkylphenols, bisphenol A (BPA), and phthalates as well as pesticides such as the organochlorines. The programming effects, on the reproductive system, after prenatal exposure to such chemicals have not been as extensively documented as for prenatal androgens and the effects of maternal nutrition. Often, effects have been observed as a result of accidental and/or inadvertent exposure to high levels of individual chemicals. However, it is becoming increasingly apparent that maternal exposure to individually low levels of mixtures of chemicals can also alter reproductive function. We will highlight the effects of a variety of different chemical exposures on the developing reproductive system and show evidence to suggest that the reproductive neuroendocrine axis may be a target for exogenous chemicals to program altered reproductive function.

6.1. Alcohol

The effects of *in utero* alcohol exposure on the reproductive system have not been documented in domestic species, such as the sheep, most available work resulting from rodent models, supplemented with data related to human exposure. In both rodents and humans, prenatal alcohol exposure has been linked with abnormalities in both males and female reproduction. In male rodents, effects include reduction in testes, prostate and seminal vesicle weight, abnormal sexual behavior [97], and delayed spermatogenesis [98]. Whereas in men, the timing of puberty is reported to be unaffected, [99,100], but exposure can result in a subsequent reduction in sperm concentrations [101]. In both female rodents [102–104] and women [105], it has been reported that puberty is delayed after prenatal alcohol exposure.

6.1.1. Does gestational alcohol exposure program the reproductive neuroendocrine system?

The fact that neuroendocrine systems are affected is strongly suggested by the changes in the timing of puberty in the female, and the observation that both the prenatal and postnatal surges of testosterone are suppressed in male rats as a result of prenatal alcohol exposure [106–108]. This hypothesis is supported by the observation that GnRH neuronal morphology is altered after prenatal alcohol exposure [104], and that both the secretion of pulsatile and the preovulatory surge of LH are affected [109–112]. As noted previously, kisspeptin has been identified as a key regulatory element within the reproductive neuroendocrine system, and recent studies have shown that prenatal alcohol exposure significantly alters the responsiveness of the kisspeptin system to estradiol and potentially progesterone (when estradiol is also present) feedback in the female [113].

6.2. Environmental chemicals

A wide range of ECs are classified as endocrine-disrupting compounds (EDCs) by virtue of the fact that they interfere with normal hormonal processes [114]. It is increasingly recognized that prenatal exposure to such EDCs can affect development and adult function of the reproductive system in laboratory animals [115–122], domestic animals [123], and humans [124,125].

Developmental exposure to EDCs have been reported to be associated with a variety of alterations of reproductive function including earlier puberty and abnormal cycles in female rats (polychlorinated bisphenols [126]), reduced testicular steroidogenesis and spermatogenesis in male rats (PCB 126 and 169 [127]), lowered prepubertal LH concentration and delayed puberty and increased luteal phase progesterone concentrations in female goats (PCB 153 [128]), advanced puberty in female lambs (octylphenol

[129]), delayed puberty in male goats (PCB153 [130]), and seminiferous tubule atrophy and reduced sperm density in male sheep (organochlorine pesticides [131]).

A number of our studies have specifically addressed effects of developmental EDC exposure on the hypothalamo-pituitary-gonadal axis in the sheep. Initial work looked at the effects of exposure to single chemicals such as octylphenol ([129] Sweeney et al., 2007) and BPA [132]. These studies documented negative effects of *in utero* exposure to octylphenol on testis development [57] and reduced semen quality [133]. The changes in testicular development were associated with lowered fetal FSH concentrations [58] although in the adult, FSH secretion was not significantly different from that in the controls [133]. Maternal BPA exposure was also found to be associated with changes in fetal gonadotrophin secretion, but in this instance, it was LH secretion that was reduced [132]. Although such single-compound studies can be informative, mixed EC exposure more closely resembles what we, domestic, and wild animals are exposed to, on a daily basis. Thus, our more recent work has concentrated on the effects of exposure to mixtures of low levels of chemicals, using an ovine model whereby sheep are exposed to ECs by grazing pasture treated with human biosolids that are a by-product of waste water treatment. Critically, the concentrations of individual chemicals in biosolids are below the published "no observed adverse effect levels" [134–136] and are, thus, not considered a risk to health. However, evidence suggests that mixtures of chemicals that are individually below the no observed adverse effect levels can have additive effects [137]. Using this model, we have collected evidence that biosolid-exposed male fetuses exhibit reduced numbers of Leydig and Sertoli cells and reduced circulating testosterone concentrations [138], and female fetuses exhibit altered ovarian development [139,140]. Later studies have indicated that these effects are not limited to the fetus, as in adults exposed to biosolids *in utero*, there is a reduction in testicular germ cell numbers in the male [141] and decreased ovarian follicle health and altered gene transcription within the adult ovarian transcriptome (Prof. P.A. Fowler, unpublished text, 2016).

6.2.1. Does gestational EC exposure program the reproductive neuroendocrine system?

These gonadal changes are accompanied by changes in the phenotype of the pituitary cell populations and a reduction in gene and protein expression within components of the GnRH neurosecretory system including GnRH itself, galanin but more importantly kisspeptin, the mRNA expression levels of which were reduced in both biosolids-exposed male and female fetuses [142,143].

7. Conclusion

The regulation of reproduction has evolved to be responsive to many different internal and external cues, so as to maximize fitness. This requires the collection and processing of large amounts of sensory information, which is integrated through the GnRH neurosecretory system within the hypothalamus. Neuronal systems express a degree of plasticity throughout life, but fetal development represents a particularly sensitive time when neuronal systems can be "shaped" in ways that affect later function. This process is critical to the sexual differentiation of behavior and function that we see with regard to the reproductive and other body systems. This plasticity, however, also represents a period of "risk" wherein environmental variables can alter normal development in ways that affect, and may even prevent, normal function. The exact mechanisms through which these programmed changes are brought about remain largely uncharacterized, and it is recognized that the periods of sensitivity to specific cues may differ depending on both the physiological system and species studied. Although it would appear that critical regulatory neuronal systems such as kisspeptin may commonly be affected, whether this is a direct effect or reflects upstream changes is not known. It is becoming clear, however, that programmed changes in the neuroendocrine systems regulating reproduction could play an important role in defining fertility. Recent reports, typically associated with prenatal EC exposure, have suggested that in addition to the direct effects on reproductive potential of the exposed offspring, prenatal programming, such as may be occurring within the GnRH neurosecretory system and thus affecting reproduction, might be occurring via epigenetic changes and thus could have transgenerational effects [144,145].

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